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Occurrence of Antimicrobial-Resistant *Escherichia coli* and *Salmonella enterica* in the Beef Cattle Production and Processing Continuum

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Specific concerns have been raised that third-generation cephalosporin-resistant (3GC^r) *Escherichia coli*, trimethoprim-sulfamethoxazole-resistant (COT^r) *E. coli*, 3GC^r *Salmonella enterica*, and nalidixic acid-resistant (NAL^r) *S. enterica* may be present in cattle production environments, persist through beef processing, and contaminate final products. The prevalences and concentrations of these organisms were determined in feces and hides (at feedlot and processing plant), pre-evisceration carcasses, and final carcasses from three lots of fed cattle ($n = 184$). The prevalences and concentrations were further determined for strip loins from 103 of the carcasses. 3GC^r *Salmonella* was detected on 7.6% of hides during processing and was not detected on the final carcasses or strip loins. NAL^r *S. enterica* was detected on only one hide. 3GC^r *E. coli* and COT^r *E. coli* were detected on 100.0% of hides during processing. Concentrations of 3GC^r *E. coli* and COT^r *E. coli* on hides were correlated with pre-evisceration carcass contamination. 3GC^r *E. coli* and COT^r *E. coli* were each detected on only 0.5% of final carcasses and were not detected on strip loins. Five hundred and 42 isolates were screened for extraintestinal pathogenic *E. coli* (ExPEC) virulence-associated markers. Only two COT^r *E. coli* isolates from hides were ExPEC, indicating that fed cattle products are not a significant source of ExPEC causing human urinary tract infections. The very low prevalences of these organisms on final carcasses and their absence on strip loins demonstrate that current sanitary dressing procedures and processing interventions are effective against antimicrobial-resistant bacteria.

The prevalence of bacterial infections resistant to antimicrobial therapy has been recognized as a critically important global public health concern (1–6). A 2013 U.S. Centers for Disease Control (CDC) report identified 18 antimicrobial-resistant (AMR) organisms as priority threats to human health, but it implicated antimicrobial use in animal production as a factor contributing to the incidence of only two of these AMR organisms (5). Regardless, the contribution of meat animal production, including beef production, to the occurrence of antimicrobial resistant human bacterial infections remains a prominent and contentious issue (7–12). Prioritization of AMR organisms is difficult since bacterial AMR is an ancient, natural, complex, and dynamic process (13, 14). Nonetheless, specific concerns have been raised about four AMR bacteria present in beef cattle production, processing, and finished products: nalidixic acid-resistant (NAL^r) nontyphoidal *Salmonella enterica* (nontyphoidal *S. enterica* will be referred to here as *Salmonella*), third-generation cephalosporin-resistant (3GC^r) *Salmonella*, 3GC^r *Escherichia coli*, and trimethoprim-sulfamethoxazole-resistant (COT^r) *E. coli* (5, 15–18).

Human urinary tract infections (UTIs) caused by extraintestinal pathogenic *E. coli* (ExPEC) have recently been described to have possible food-borne origins that include beef products (19–21). The folate synthesis inhibitor combination trimethoprim-sulfamethoxazole (co-trimoxazole) is the preferred therapy for UTI (22, 23), but clinicians have reported an increase in COT^r *E. coli* (24). COT^r *E. coli*, ExPEC, and COT^r ExPEC have been isolated from retail beef products (21, 25), but to our knowledge there are no published studies on COT^r *E. coli*, ExPEC, and COT^r ExPEC in beef production and processing environments.

The dynamics of 3GC^r *E. coli*, COT^r *E. coli*, 3GC^r *Salmonella*, and NAL^r *Salmonella* subpopulations in beef cattle production and processing environments have not been thoroughly investigated. Elucidation of population dynamics in cattle production and processing is important to understanding the food safety impact of antimicrobial resistance in beef production, as prevalences and concentrations of bacteria on cattle hides are strongly correlated with carcass contamination during hide removal (26–29).

The U.S. National Antimicrobial Resistance Monitoring System (NARMS) programs' surveillance of AMR food-borne pathogens in beef production is limited to culture of *Salmonella* from ground beef (sampled both at processing and at retail) and from beef carcass swabs and to culture of *E. coli* from a subset of retail ground beef samples (25, 30, 31). NARMS surveillance does not provide data required for determining the effectiveness of in-plant processing interventions or risk analysis of food-borne bacteria resistant to antimicrobials important to human medicine in production environments (feedlots) or during processing (hides and pre-evisceration carcasses prior to interventions). The objective of the present study was to begin addressing these data gaps and to determine the prevalence and concentrations of generic *Salmonella*, 3GC^r *Salmonella*, NAL^r *Salmonella*, generic *E. coli*, 3GC^r *E.*

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coli, and COT^r *E. coli* from feedlot, through processing, to final products. We define "generic *E. coli*" and "generic *Salmonella*" as all *E. coli* and all *Salmonella*, respectively, regardless of susceptibility to any antimicrobial agent or pathogenicity status. In addition, generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* isolates were screened for the presence of virulence-associated markers of human ExPEC infections (21, 32).

MATERIALS AND METHODS

Cattle populations and sampling methods. Three groups of fed beef cattle, designated lot 1, lot 2, and lot 3, were examined in the present study. Lot 1 and lot 2 were housed in the same Nebraska feedlot. Lot 3 cattle were housed at a different Nebraska feedlot. All three lots of cattle were harvested at the same beef processing plant. Seven types of samples were obtained: feedlot fecal, processing fecal, feedlot hide, processing hide, pre-evisceration carcass, final carcass, and strip loin. No attempts were made to match samples to individual animals.

Lots 1, 2, and 3 consisted of 74, 74, and 136 cattle, respectively. For lots 1 and 2, fecal and hide samples were obtained from all 74 head at the feedlot in May 2013 and June 2013, respectively. For lot 3, fecal and hide samples were obtained from 36 arbitrarily selected cattle at the feedlot in October of 2013. Cattle were transported to the processing plant and harvested 20 to 25 days after the feedlot samples were obtained. For lots 1 and 2, fecal, hide, pre-evisceration carcass (after hide removal but before any carcass sanitizing treatments), and chilled final carcass samples were obtained from all 74 carcasses. For lot 3, fecal, hide, pre-evisceration carcass, and chilled final carcass samples were obtained from 36 arbitrarily selected carcasses. For lots 1 and 2, strip loins were obtained from 51 and 52 arbitrarily selected carcasses, respectively, vacuum-sealed, and stored at 4°C. Purge was recovered these strip loins 1 week after storage. Strip loin samples could not be collected for lot 3.

Fecal samples were collected by inserting a foam-tipped swab (catalog no. 10812-022; VWR International, Buffalo Grove, IL) 3 to 5 cm into the anus of each animal. Immediately after fecal sample collection, the swab was placed into 5 ml of phosphate-buffered tryptic soy broth (TSB-PO; 30 g of TSB, 2.31 g of KH_2PO_4, and 12.54 g of K_2HPO_4 per liter, final pH 7.2; Becton Dickinson, Franklin Lakes, NJ) (33). Hide samples were collected from each animal by swabbing a 1,000-cm^2 area located behind the shoulder with a sterile sponge (Whirl Pack; Nasco, Fort Atkinson, WI) prewetted with 20-ml of buffered peptone water (BPW; Becton Dickinson). Immediately after hide sampling, the sponge was placed into a sterile bag. Feedlot fecal and hide samples were obtained while cattle were restrained in a squeeze chute at the feedlot. Processing plant fecal and hide samples were obtained at the processing plant immediately following exsanguination, prior to a hide wash cabinet.

Pre-evisceration and final carcass samples were obtained by swabbing an ~4,000-cm^2 area from brisket to foreshank on a carcass half with a sterile sponge (Whirl Pak) prewetted with 20 ml of BPW. Immediately following carcass sampling, the sponge was placed into a sterile bag. Pre-evisceration carcass samples were obtained after hide removal, prior to the application of antimicrobial carcass decontamination steps. Final carcass samples were obtained from carcasses chilled in the cooler overnight.

Strip loin samples were obtained by aseptically pipetting up to 26-ml of purge, the liquid that forms within the package, from each individually vacuum-sealed package containing a strip loin into a 50-ml conical test tube.

Sample processing. For hide and carcass samples, sponges were homogenized by hand massage in the sample bags for 15 s, and a 1-ml enumeration aliquot was removed (see Fig. S1 and S2 in the supplemental material). First, 80 ml of TSB-PO was added to each sample, followed by incubation at 25°C for 2 h and 42°C for 6 h and then held at 4°C until secondary enrichments were performed the following day (33–35). Fecal samples were suspended by vortexing for 30 s, and then a 1-ml enumeration aliquot was removed (see Fig. S3 in the supplemental material). The remaining fecal sample was then incubated at 42°C for 8 h and then held at 4°C until secondary enrichments were performed the following day. Strip loin purge samples were vortexed for 30 s, and a 1-ml enumeration aliquot was removed (see Fig. S4 in the supplemental material). The remaining purge was combined with TSB-PO in a 1:10 ratio (e.g., 25 ml of purge was combined with 225 ml of TSB-PO) and incubated at 25°C for 2 h and 42°C for 6 h and then held at 4°C until secondary enrichments were performed the following day.

Generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* enumeration. For fecal, hide, and pre-evisceration carcass samples, generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* were each enumerated by spiral plating using a Spiral plater (Spiral Biotech, Norwood, MA), with 50-μl aliquots of appropriate dilutions of the enumeration aliquoted onto CHROMagar *E. coli* (CEC) plates (DRG International, Inc., Springfield, NJ), CEC plates supplemented with 2 mg liter^{-1} of cefotaxime (CEC+CTX), and CEC plates supplemented with 4 mg liter^{-1} of trimethoprim and 76 mg liter^{-1} sulfamethoxazole (CEC+COT), respectively (see Fig. S1 and S3 in the supplemental material). All antimicrobials were obtained from Sigma-Aldrich Corp., St. Louis, MO, unless otherwise stated. Supplementation of media with cefotaxime to enumerate 3GC^r *E. coli* has been described previously (36, 37). The concentrations of trimethoprim and sulfamethoxazole added to CEC were set to match the resistance breakpoint for COT^r *E. coli* described in the most recently available NARMS Retail Meat Report (25). Plates were incubated overnight at 37°C. Blue colonies on CEC, CEC+CTX, and CEC+COT were enumerated as presumptive generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli*, respectively. From each plate up to two presumptive colonies were inoculated into TSB and incubated overnight at 37°C. Then, an aliquot of overnight culture was removed, combined with BAX lysis buffer (DuPont Qualicon, Inc., Wilmington, DE) and incubated according to the manufacturer's instructions to generate template DNA for molecular assays. An aliquot of each DNA lysate was used to confirm the presumptive colonies as *E. coli* by multiplex PCR for the presence of *lacY*, *lacZ*, *cyd*, and *uidA* genes (38). Glycerol was added to each overnight TSB culture to a final concentration of 15% and preserved at −20°C. Colony DNA lysates were preserved at −20°C.

Generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* enumeration. For fecal, hide, and pre-evisceration carcass samples generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* were each enumerated by spiral plating, using a Spiral plater (Spiral Biotech), 50-μl aliquots of the enumeration aliquot onto xylose-lysine-desoxycholate agar (Remel, Inc., Lenexa, KS) plates supplemented with 4.6 mg liter^{-1} tergitol (also known as niaproof), 15 mg liter^{-1} novobiocin, and 5 mg liter^{-1} cefesulodin (XLD_{tnc}) (35), xylose-lysine-desoxycholate agar plates supplemented with 2 mg liter^{-1} cefotaxime (XLD+CTX), and xylose-lysine-desoxycholate agar plates supplemented with 32 mg liter^{-1} nalidixic acid (XLD+NAL), respectively (see Fig. S1 and S3 in the supplemental material). *Salmonella* enumeration by direct plating onto XLD_{tnc} was described previously (35). The concentration of cefotaxime used in XLD+CTX plates was based on the recommendations by the European Food Safety Authority (39). The concentration of nalidixic acid used to supplement XLD media was based on the resistance breakpoint for NAL^r *Salmonella* described in the most recently available NARMS retail meat report (25). Plates were incubated overnight at 37°C and then held at 25°C for up to 72 h to allow H_2S production and black color development. Black colonies on XLD_{tnc}, XLD+CTX, and XLD+NAL plates were counted as presumptive generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella*, respectively. For confirmation, from each plate up to two presumptive colonies were inoculated into TSB and incubated overnight at 37°C. From each overnight TSB culture, an aliquot was removed and combined with BAX lysis buffer, followed by incubation according to the manufacturer's instructions to generate template DNA for molecular assays. An aliquot of each DNA lysate was used to confirm the presumptive colonies as *Salmonella* by PCR for the *invA* gene (40, 41). Glycerol was added to each overnight TSB culture to a final concentration of 15% and preserved at −20°C. Colony DNA lysates were preserved at −20°C.

Generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* prevalences. For all samples secondary enrichments were prepared by combining 0.5-ml aliquots of enrichment with 2.5 ml of MacConkey (MAC) broth (Becton Dickinson), 2.5 ml of MAC broth supplemented with 2.4 mg liter^{-1} cefotaxime (MAC+CTX), and 2.5-ml of MAC broth supplemented with 4.8 mg liter^{-1} trimethoprim and 91.2 mg liter^{-1} sulfamethoxazole (MAC+COT). Secondary enrichments were and incubated overnight at 42°C. MAC, MAC+CTX, and MAC+COT secondary enrichments were then struck onto CEC, CEC+CTX, and CEC+COT plates, respectively, followed by incubation overnight at 37°C (see Fig. S1, S2, S3, and S4 in the supplemental material). Blue colonies on CEC, CEC+CTX, and CEC+COT plates were considered presumptive generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli*, respectively. From each plate up to two presumptive colonies were inoculated into TSB and incubated overnight at 37°C. Presumptive *E. coli* was PCR confirmed as *E. coli* and preserved as described above.

Generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* prevalences. For all samples, 1 ml of enrichment was combined with 20 μl of *Salmonella*-specific immunomagnetic separation beads (Life Technologies, Grand Island, NY) (42). The bacterium-bead complex was extracted, placed into Rappaport-Vassiliadis soy peptone broth (RVS; Remel), and incubated at 42°C overnight (see Fig. S1, S2, S3, and S4 in the supplemental material). The RVS selective enrichment was then swabbed onto XLD_{tnc}, XLD+CTX, and XLD+NAL plates. Plates were incubated overnight at 37°C and then held at 25°C for up to 72 h to allow H_2S production and black color development. From each plate, up to two presumptive *Salmonella* colonies were selected for confirmation by PCR for the presence of the *Salmonella*-specific portion of the *invA* gene as described above.

Enumeration of final carcass and strip loin samples. For final carcass and strip loin samples, the 1-ml enumeration aliquots were applied to PetriFilm EB plates (3M Microbiology, St. Paul, MN) and were incubated according to the manufacturer's instructions (see Fig. S2 and S4 in the supplemental material). Plates were then held at 4°C to await prevalence results. PetriFilm EB plates corresponding to samples found to be prevalent for generic *E. coli*, 3GC^r *E. coli*, COT^r *E. coli*, generic *Salmonella*, 3GC^r *Salmonella*, or NAL^r *Salmonella* was replica plated onto CEC, CEC+CTX, CEC+COT, XLD_{tnc}, XLD+CTX, or XLD+NAL plates, respectively. Plates were incubated and enumerated, selected colonies were grown overnight, lysates were prepared, lysates were PCR confirmed, and overnight growth was preserved as described above.

Calculation of sample concentrations. Fecal sample enumeration plate counts were converted to log CFU/swab values. The lower limit of enumeration from fecal samples was 2.00 log CFU/swab, and the theoretical lower limit of prevalence detection was 0.00 log CFU/swab. Fecal samples with no confirmed colonies on enumeration plates, but with confirmed colonies on prevalence plates were assumed to have a concentration between 0.00 and 1.99 log CFU/swab.

Hide sample enumeration plate counts were converted to log CFU/100-cm^2 values. The lower limit of enumeration from hide samples was 1.60 log CFU/100 cm^2, and the theoretical lower limit of prevalence detection was −1.00 log CFU/100 cm^2. Hide samples with no confirmed colonies on enumeration plates but with confirmed colonies on prevalence plates were assumed to have a concentration between −1.00 and 1.60 log CFU/100 cm^2.

Pre-evisceration enumeration plate counts were converted to log CFU/100-cm^2 values. The lower limit of enumeration from pre-evisceration carcass samples was 1.00 log CFU/100 cm^2, and the theoretical lower limit of prevalence detection was −1.60 log CFU/100 cm^2. Pre-evisceration carcass samples with no confirmed colonies on enumeration plates but with confirmed colonies on prevalence plates were assumed to have a concentration between −1.60 and 0.99 log CFU/100 cm^2.

Final carcass enumeration plate counts were converted to log CFU/100-cm^2 values. The lower limit of enumeration from final carcass samples was −0.30 log CFU/100 cm^2, and the theoretical lower limit of prevalence detection was −1.60 log CFU/100 cm^2. Final carcass samples with no confirmed colonies on enumeration plates, but with confirmed colonies on prevalence plates were assumed to have a concentration between −1.60 and −0.31 log CFU/100 cm^2.

Strip loin purge enumeration plate counts were converted to log CFU/ml values. The lower limit of enumeration from strip loin purge was 0.00 log CFU/ml, and the theoretical lower limit of prevalence detection was −1.40 log CFU/ml. Strip loin purge samples with no confirmed colonies on enumeration plates but with confirmed colonies on prevalence plates were assumed to have a concentration between −1.40 and −0.01 log CFU/ml.

Genotyping and antimicrobial susceptibility testing of generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* isolates. Samples were grouped by lot (1, 2, or 3) and sample type (feedlot fecal, processing fecal, feedlot hide, processing hide, pre-evisceration carcass, final carcass, and purge from strip loin). For each group the 12 samples with the highest concentrations of generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* were selected. When there were fewer than 12 samples in a group with enumerable concentrations, the remaining selections were arbitrarily made from samples that were prevalence positive but not enumerable. For each generic *E. coli*, 3GC^r *E. coli*, or COT^r *E. coli* sample selected a confirmed colony was struck onto a CEC, CEC+CTX, or CEC+COT plate, respectively. Plates were incubated at 37°C overnight, and then a single isolated blue colony was selected and streaked onto a Trypticase soy agar (TSA) plate (Becton Dickinson), followed by incubation at 37°C overnight. One isolated colony from each TSA plate was inoculated into a 0.7-ml TSB culture and incubated overnight at 37°C. An aliquot of overnight culture was then removed, combined with BAX lysis buffer, and incubated according to the manufacturer's instructions to generate template DNA for molecular assays. Glycerol was added to each overnight TSB culture to a final concentration of 15% and preserved at −80°C. Isolate DNA lysates were preserved at −20°C. Isolate DNA lysates were used to reconfirm the isolates were *E. coli* by multiplex PCR for the presence of *lacY*, *lacZ*, *cyd*, and *uidA* genes (38). Isolate DNA lysates were also tested with a multiplex PCR to determine the presence of five markers associated with ExPEC virulence, *afa/dra*, *iutA*, *kpsMT* II, *papA*, and *papC* (*papA* and *papC* were amplified separately but analyzed together as a single marker), and *sfa/foc*, using the primers and conditions described by Xia et al. (21). Isolates harboring two or more virulence markers were classified as ExPEC (21, 43).

3GC^r *E. coli* isolate DNA lysates were subjected to PCR using the primers and conditions described by Kozak et al. (44) and Cottell et al. (45) to determine the presence of bla_{CMY} genes and $bla_{CTX\text{-}M}$ genes, respectively. COT^r *E. coli* isolate DNA lysates were subjected to PCR using the primers and conditions described by Kozak et al. (44) to determine the presence of *sul1*, *sul2*, and *sul3* genes. COT^r *E. coli* isolate DNA lysates were subjected to PCR using the primers and conditions described by Grape et al. (46) to determine the presence of *dfrA1*, *dfrA5*, *dfrA7/dfrA17*, and *dfrA12* genes.

Antimicrobial susceptibility testing was performed with 3GC^r *E. coli* and COT^r *E. coli* isolates using the Sensititre broth microdilution system and CMV2AGNF plates (TREK Diagnostic Systems, Cleveland, OH) to determine the MICs for each of 15 antimicrobial agents. The following organisms were used as quality control strains in the antimicrobial sensitivity assays: *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923. The antimicrobials and breakpoints for resistance in this panel were as follows: amoxicillin and clavulanic acid (AMC), ≥32 and ≥16 μg ml^{-1}, respectively; ampicillin (AMP), ≥32 μg ml^{-1}; azithromycin (AZI), ≥32 μg ml^{-1}; cefoxitin (FOX), ≥32 μg ml^{-1}; ceftiofur (TIO), ≥8 μg ml^{-1}; ceftriaxone (AXO), ≥4 μg ml^{-1}; chloramphenicol (CHL), ≥32 μg ml^{-1}; ciprofloxacin (CIP), ≥4 μg ml^{-1}; gentamicin (GEN), ≥16 μg ml^{-1}; kanamycin (KAN), ≥64 μg ml^{-1}; nalidixic acid (NAL), ≥32 μg ml^{-1}; streptomycin (STR), ≥64 μg ml^{-1}; sulfisoxazole (FIS), ≥512 μg ml^{-1}; tetracycline (TET), ≥16 μg ml^{-1}; and trimethoprim and sulfamethoxazole (COT), ≥4 and ≥76 μg ml^{-1}, respectively. Antimicrobial breakpoints and three-letter abbreviations were as described in the most recently available NARMS retail meat report (25).

TABLE 1 Prevalences and concentrations of *E. coli* and *Salmonella* in fecal samples obtained at feedlots

Organism and lot[a]	No. sampled	% prevalence	Frequency of fecal samples with indicated concn (log CFU/swab)						
			0.00–1.99	2.00–2.99	3.00–3.99	4.00–4.99	5.00–5.99	6.00–6.99	7.00–7.99
Generic *E. coli*	184	100.0	0	0	0	11	50	120	3
Lot 1	74	100.0	0	0	0	0	10	64	0
Lot 2	74	100.0	0	0	0	0	19	52	3
Lot 3	36	100.0	0	0	0	11	21	4	0
3GC^r *E. coli*	184	82.6	114	32	6	0	0	0	0
Lot 1	74	60.8	33	12	0	0	0	0	0
Lot 2	74	98.6	55	13	5	0	0	0	0
Lot 3	36	94.4	26	7	1	0	0	0	0
COT^r *E. coli*	184	98.4	81	52	40	7	1	0	0
Lot 1	74	98.6	18	24	26	5	0	0	0
Lot 2	74	100.0	39	20	12	2	1	0	0
Lot 3	36	94.4	24	8	2	0	0	0	0
Generic *Salmonella*	184	5.4	7	3	0	0	0	0	0
Lot 1	74	0.0	0	0	0	0	0	0	0
Lot 2	74	0.0	0	0	0	0	0	0	0
Lot 3	36	27.8	7	3	0	0	0	0	0
3GC^r *Salmonella*	184	0.5	1	0	0	0	0	0	0
Lot 1	74	0.0	0	0	0	0	0	0	0
Lot 2	74	0.0	0	0	0	0	0	0	0
Lot 3	36	2.8	1	0	0	0	0	0	0
NAL^r *Salmonella*	184	0.0	0	0	0	0	0	0	0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.

Genotyping and antimicrobial susceptibility testing of generic *Salmonella* isolates, 3GC^r *Salmonella*, and NAL^r *Salmonella* isolates. Samples were grouped by lot (1, 2, or 3) and sample type (feedlot fecal, processing fecal, feedlot hide, processing hide, and pre-evisceration carcass). For each group, the 12 samples with the highest concentrations of generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* were selected. When there were fewer than 12 samples in a group with enumerable concentrations, the remaining selections were arbitrarily made from samples that were prevalence positive but not enumerable. For each generic *Salmonella*, 3GC^r *Salmonella*, or NAL^r *Salmonella* sample selected a confirmed colony was struck onto an XLD_{tnc}, XLD+CTX, or XLD+NAL plate, respectively. Plates were incubated at 37°C overnight, and then a single isolated black colony was selected, streaked onto a TSA plate, and incubated at 37°C overnight. One isolated colony from each TSA plate was inoculated into a 0.7-ml TSB culture, followed by incubation overnight at 37°C. An aliquot of overnight culture was then removed, combined with BAX lysis buffer, and incubated according to the manufacturer's instructions to generate template DNA for molecular assays. Glycerol was added to each overnight TSB culture to a final concentration of 15% and preserved at −80°C. Isolate DNA lysates were used to reconfirm the isolates were *Salmonella* by PCR for the presence of the *invA* gene (40, 41). 3GC^r *Salmonella* isolate DNA lysates were subjected to PCR using the primers and conditions described by Kozak et al. (44) and Cottell et al. (45) to determine the presence of bla_{CMY} and $bla_{CTX\text{-}M}$ genes, respectively.

Antimicrobial susceptibility testing was performed using the Sensititre broth microdilution system and CMV2AGNF plates as described above except the resistance breakpoint for CIP was lowered to ≥1 μg ml^{-1} as described for *Salmonella* in the most recently available NARMS retail meat report (25).

ExPEC virulence-associated genotyping of generic, COT^r, and 3GC^r *E. coli* colony confirmation lysates from carcass and strip loin samples. Confirmed generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* colony DNA lysates from pre-evisceration carcass, final carcass, and strip loin samples were examined for the presence of five ExPEC markers associated with virulence and interpreted as described above. Samples with at least one ExPEC colony were considered ExPEC prevalent.

RESULTS

Fecal samples obtained at feedlots. Generic *E. coli* was present in 100% of the fecal samples obtained at feedlots (Table 1). Concentrations of generic *E. coli* ranged from 4.00 to 7.54 log CFU/swab; however, 94.0% of the samples contained generic *E. coli* concentrations ≥5.00 log CFU/swab. 3GC^r *E. coli* prevalence in fecal samples obtained at feedlots was 82.6%. 3GC^r *E. coli* was present at concentrations up to 3.62 log CFU/swab, but concentrations were between 0.00 and 1.99 log CFU/swab in 62.0% of feedlot fecal samples. The COT^r *E. coli* fecal prevalence at feedlots was 98.4%. COT^r *E. coli* was detected at concentrations up to 5.39 log CFU/swab, but the COT^r *E. coli* concentrations were between 0.00 and 3.99 log CFU/swab in 94.0% of feces.

Generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* were detected in 5.4, 0.5, and 0.0% of fecal samples obtained at feedlots, respectively (Table 1). Only feedlot fecal samples obtained from lot 3 cattle contained *Salmonella*. *Salmonella* was present at concentrations of up to 2.00 log CFU/swab (Table 1).

Hides sampled at feedlots. Prevalences of generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* on hides at feedlots were 100.0, 89.1, and 100.0%, respectively (Table 2). Generic *E. coli* concentrations on hides at feedlots ranged between 3.53 and 6.90 log CFU/100 cm^2, but 94.0% of the hides harbored generic *E. coli* at concentrations between 4.00 and 5.99 log CFU/100 cm^2. 3GC^r *E. coli* was detected

TABLE 2 Prevalences and concentrations of *E. coli* and *Salmonella* on hides at feedlots

Organism and lot[a]	No. sampled	% prevalence	Frequency of hides with indicated concn (log CFU/100 cm^2)						
			−1.00 to 1.59	1.60–2.99	3.00–3.99	4.00–4.99	5.00–5.99	6.00–6.99	7.00–7.99
Generic *E. coli*	184	100.0	0	0	3	41	132	8	0
Lot 1	74	100.0	0	0	0	4	70	0	0
Lot 2	74	100.0	0	0	0	18	48	8	0
Lot 3	36	100.0	0	0	3	19	14	0	0
3GC^r *E. coli*	184	89.1	162	2	0	0	0	0	0
Lot 1	74	81.1	60	0	0	0	0	0	0
Lot 2	74	91.9	66	2	0	0	0	0	0
Lot 3	36	100.0	36	0	0	0	0	0	0
COT^r *E. coli*	184	100.0	142	35	7	0	0	0	0
Lot 1	74	100.0	42	25	7	0	0	0	0
Lot 2	74	100.0	64	10	0	0	0	0	0
Lot 3	36	100.0	36	0	0	0	0	0	0
Generic *Salmonella*	184	26.1	48	0	0	0	0	0	0
Lot 1	74	8.1	6	0	0	0	0	0	0
Lot 2	74	8.1	6	0	0	0	0	0	0
Lot 3	36	100.0	36	0	0	0	0	0	0
3GC^r *Salmonella*	184	10.9	20	0	0	0	0	0	0
Lot 1	74	0.0	0	0	0	0	0	0	0
Lot 2	74	0.0	0	0	0	0	0	0	0
Lot 3	36	55.6	20	0	0	0	0	0	0
NAL^r *Salmonella*	184	0.0	0	0	0	0	0	0	0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.

on hides sampled at feedlots at concentrations up to 2.78 log CFU/100 cm^2, but 88.0% of the hides had 3GC^r *E. coli* concentrations that were between −1.00 and 1.59 log CFU/100 cm^2. COT^r *E. coli* was detected on hides sampled at feedlots at concentrations up to 3.89 log CFU/100 cm^2; however, 77.2% of hides had COT^r *E. coli* concentrations between −1.00 and 1.59 log CFU/100 cm^2.

Generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* were detected on 26.1, 10.9, and 0.0% of hides sampled at feedlots, respectively (Table 2). No sample contained either generic or 3GC^r *Salmonella* at concentrations ≥1.60 log CFU/100 cm^2. Generic *Salmonella* prevalence on hides at feedlots varied by lot, with prevalences of 8.1, 8.1, and 100.0% observed for lot 1, lot 2, and lot 3, respectively. 3GC^r *Salmonella* was not detected on lot 1 or lot 2 hides but was detected on 55.6% of the lot 3 hides.

Fecal samples obtained at processing. Prevalences of generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* in fecal samples at processing were 100.0, 75.0, and 95.1%, respectively (Table 3). Generic *E. coli* was present at concentrations between 4.00 and 7.87 log CFU/swab, but in 86.4% of the samples the concentrations were ≥5.00 log CFU/swab. 3GC^r *E. coli* was present in fecal samples at processing at concentrations up to 2.90 log CFU/swab, but in 63.0% of the samples the concentrations were between 0.00 and 1.99 log CFU/swab. Fecal samples obtained at processing contained COT^r *E. coli* at concentrations up to 4.76 log CFU/swab, but in 80.4% of the samples the COT^r *E. coli* concentrations were between 0.00 and 2.99 log CFU/swab.

Generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* prevalences were 44.6, 1.6, and 0.0% in fecal samples obtained at processing, respectively (Table 3). The highest concentration of *Salmonella* in a fecal sample obtained at processing was 2.78 log CFU/swab. Generic *Salmonella* prevalences for lot 1, lot 2, and lot 3 were 20.3, 63.5, and 55.6%, respectively. All three processing fecal samples with 3GC^r *Salmonella* were from lot 3.

Hides sampled at processing. Generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* were each present on 100% of hides sampled at processing (Table 4). Generic *E. coli* concentrations on hides at processing ranged between 4.00 and 7.04 log CFU/100 cm^2, but the distribution of the concentrations varied by lot. During processing 94.6% of the lot 1 cattle hides had generic *E. coli* concentrations between 6.00 and 7.04 log CFU/100 cm^2, but 99.1% of the lot 2 and lot 3 hides had generic *E. coli* concentrations between 4.00 and 5.99 log CFU/100 cm^2.

3GC^r *E. coli* concentrations on cattle hides at processing ranged from −1.00 to 3.53 log CFU/100 cm^2 (Table 4). Lot differences in the distribution of 3GC^r *E. coli* concentrations were observed. The percentages of hides with 3GC^r *E. coli* concentrations of ≥1.60 log CFU/100 cm^2 were 81.1, 17.6, and 22.2% for lot 1, lot 2, and lot 3, respectively.

COT^r *E. coli* concentrations on hides at processing ranged between −1.00 and 4.56 log CFU/100 cm^2 (Table 4). Lot differences in the distribution of the COT^r *E. coli* concentrations were observed. COT^r *E. coli* hide concentrations of ≥4.00 log CFU/100 cm^2 were 25.7, 6.8, and 0.0% for lot 1, lot 2, and lot 3, respectively.

Generic *Salmonella* was present on 99.5% of hides at processing with concentrations up to 2.98 log CFU/100 cm^2 (Table 4). However, hides with concentrations of generic *Salmonella* ≥1.60 log CFU/100 cm^2 varied by lot: 10.8% for lot 1, 83.8% for lot 2, and 11.1% for lot 3. 3GC^r *Salmonella* was present on 14 hides (one lot 1 hide and 13 lot 3 hides) and concentrations were never ≥1.60 log CFU/100 cm^2. NAL^r *Salmonella* was detected on one hide.

TABLE 3 Prevalences and concentrations of *E. coli* and *Salmonella* in fecal samples obtained at processing

Organism and lot[a]	No. sampled	% prevalence	Frequency of fecal samples with indicated concn (log CFU/swab)						
			0.00–1.99	2.00–2.99	3.00–3.99	4.00–4.99	5.00–5.99	6.00–6.99	7.00–7.99
Generic *E. coli*	184	100.0	0	0	0	25	84	59	16
Lot 1	74	100.0	0	0	0	0	15	45	14
Lot 2	74	100.0	0	0	0	7	52	13	2
Lot 3	36	100.0	0	0	0	18	17	1	0
3GC^r *E. coli*	184	75.0	116	22	0	0	0	0	0
Lot 1	74	64.9	41	7	0	0	0	0	0
Lot 2	74	81.1	53	7	0	0	0	0	0
Lot 3	36	83.3	22	8	0	0	0	0	0
COT^r *E. coli*	184	95.1	81	67	24	3	0	0	0
Lot 1	74	98.6	29	26	15	3	0	0	0
Lot 2	74	98.6	27	37	9	0	0	0	0
Lot 3	36	80.6	25	4	0	0	0	0	0
Generic *Salmonella*	184	44.6	74	8	0	0	0	0	0
Lot 1	74	20.3	15	0	0	0	0	0	0
Lot 2	74	63.5	45	2	0	0	0	0	0
Lot 3	36	55.6	14	6	0	0	0	0	0
3GC^r *Salmonella*	184	1.6	2	1	0	0	0	0	0
Lot 1	74	0.0	0	0	0	0	0	0	0
Lot 2	74	0.0	0	0	0	0	0	0	0
Lot 3	36	8.3	2	1	0	0	0	0	0
NAL^r *Salmonella*	184	0.0	0	0	0	0	0	0	0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.

Pre-evisceration carcasses. Prevalences of generic *E. coli* on pre-evisceration carcasses were 100.0, 77.0, and 97.2% for lot 1, lot 2, and lot 3, respectively (Table 5). Percentages of pre-evisceration carcasses with generic *E. coli* concentrations between 1.00 and 2.00 log CFU/100 cm^2 were 36.5, 1.4, and 0.0% for lot 1, lot 2, and lot 3, respectively.

3GC^r *E. coli* and COT^r *E. coli* were present on only 2.7 and 32.6% of pre-evisceration carcasses, respectively, and their concentrations on pre-evisceration carcasses were never >0.99 log CFU/100 cm^2 (Table 5). The prevalences of COT^r *E. coli* on pre-evisceration carcasses were 77.0, 2.7, and 2.8% for lot 1, lot 2, and lot 3, respectively.

Generic *Salmonella* was present on four pre-evisceration carcasses, all from lot 3 (Table 5). 3GC^r *Salmonella* and NAL^r *Salmonella* were not detected on any pre-evisceration carcass.

Final carcasses. Generic *E. coli* prevalences on final carcasses were 91.9, 5.4, and 52.8% for lot 1, lot 2, and lot 3, respectively (Table 6). Concentrations of generic *E. coli* on final carcasses ranged from −1.60 to 1.10 log CFU/100 cm^2, but generic *E. coli* concentrations of ≥−0.30 log CFU/100 cm^2 were present on lot 1 final carcasses. 3GC^r *E. coli* was detected on only one final carcass, from lot 1. COT^r *E. coli* was also detected on one final carcass, also from lot 1. *Salmonella* was not detected on final carcasses (Table 6).

Strip loins. Generic *E. coli* prevalences were 86.5% in purge samples obtained from 52 lot 1 strip loins and 86.2% in purge samples obtained from 51 lot 2 strip loins. Concentrations of generic *E. coli* in purge from strip loins ranged from −1.40 to 1.30 log CFU/ml but were below the limit of enumeration (<0.00 log CFU/ml) in 98 (95.1%) samples. Concentrations in the five enumerable samples were 0.00, 0.00, 0.00, 0.30, and 1.30 log CFU/ml. Strip loins from lot 3 were not available for microbial testing. 3GC^r *E. coli*, COT^r *E. coli*, generic *Salmonella*, 3GC^r *Salmonella*, and NAL^r *Salmonella* were not detected in the purge obtained from any strip loin sampled in the present study.

Antimicrobial susceptibilities, the presence of β-lactamase genes, and the presence of ExPEC virulence markers in 3GC^r *E. coli* isolates. All 150 3GC^r *E. coli* isolates were resistant to AMP, TIO, and AXO (Table 7). bla_{CMY} was present in 96 (64.0%) isolates, including one isolate in which bla_{CTX-M} was also present. All 96 isolates with bla_{CMY} were resistant to AUG and FOX. bla_{CTX-M} was present without the presence of bla_{CMY} in 54 (36.0%) isolates; none of these isolates were resistant to FOX, and only one was resistant to AUG.

None of the 3GC^r *E. coli* isolates were classified has ExPEC since no isolate harbored more than one virulence marker (Table 8). Only one isolate possessed the *iutA* virulence marker; none of the other virulence markers were detected from the other 149 3GC^r *E. coli* isolates.

Antimicrobial susceptibilities, presence of *dfrA* genes, the presence of *sul* genes, and the presence of ExPEC virulence markers in COT^r *E. coli* isolates. All 160 COT^r *E. coli* isolates examined were COT^r and FIS^r (Table 7). The *sul1*, *sul2*, and *sul3* genes were detected in 70.6, 39.4, and 22.5% of isolates, respectively. All three *sul* genes were detected in 9.4% of isolates, two *sul* genes were detected in 14.4% of isolates, one *sul* gene was detected in 75.6% of isolates, and no *sul* gene was detected in 0.6% of isolates. The *dfrA1*, *dfrA5*, *dfrA7/dfrA17*, and *dfrA12* genes were detected in 10.0, 51.3, 31.3, and 17.5% of isolates, respectively. Three *dfrA* genes were detected in 4.4% of isolates, two *dfrA* genes

TABLE 4 Prevalences and concentrations of *E. coli* and *Salmonella* on hides at processing

Organism and lot[a]	No. sampled	% prevalence	Frequency of hides with indicated concn (log CFU/100 cm^2)						
			–1.00 to 1.59	1.60–2.99	3.00–3.99	4.00–4.99	5.00–5.99	6.00–6.99	7.00–7.99
Generic *E. coli*	184	100.0	0	0	0	45	68	69	2
Lot 1	74	100.0	0	0	0	0	4	68	2
Lot 2	74	100.0	0	0	0	16	57	1	0
Lot 3	36	100.0	0	0	0	29	7	0	0
3GC^r *E. coli*	184	100.0	103	69	12	0	0	0	0
Lot 1	74	100.0	14	49	11	0	0	0	0
Lot 2	74	100.0	61	13	0	0	0	0	0
Lot 3	36	100.0	28	7	1	0	0	0	0
COT^r *E. coli*	184	100.0	32	23	105	24	0	0	0
Lot 1	74	100.0	1	5	49	19	0	0	0
Lot 2	74	100.0	0	13	56	5	0	0	0
Lot 3	36	100.0	31	5	0	0	0	0	0
Generic *Salmonella*	184	99.5	109	74	0	0	0	0	0
Lot 1	74	100.0	66	8	0	0	0	0	0
Lot 2	74	100.0	12	62	0	0	0	0	0
Lot 3	36	97.2	31	4	0	0	0	0	0
3GC^r *Salmonella*	184	7.6	14	0	0	0	0	0	0
Lot 1	74	1.4	1	0	0	0	0	0	0
Lot 2	74	0.0	0	0	0	0	0	0	0
Lot 3	36	36.1	12	1	0	0	0	0	0
NAL^r *Salmonella*	184	0.5	1	0	0	0	0	0	0
Lot 1	74	1.4	1	0	0	0	0	0	0
Lot 2	74	0.0	0	0	0	0	0	0	0
Lot 3	36	0.0	0	0	0	0	0	0	0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.

were detected in 10.0% of isolates, one *dfrA* gene was detected in 76.9% of isolates, and none of the *dfrA* genes screened for were detected in 8.8% of isolates.

Two (1.3%) of the 160 COT^r *E. coli* isolates were identified as ExPEC (Table 8). Both ExPEC isolates were obtained from hides at processing, one each from lot 2 and lot 3. Each of the ExPEC isolates contained the *iutA* and *papC* makers. The *iutA* virulence-associated factor was detected from 50.6% of the COT^r *E. coli* isolates and was present in isolates obtained from each lot. The *papC* gene was detected from only the two ExPEC isolates. The *afa*/*dra*, *sfa*/*foc*, and *papA* virulence-associated factors were not present in any of the COT^r *E. coli* isolates.

Presence of ExPEC virulence markers in generic *E. coli* isolates. None of the 232 generic *E. coli* isolates were ExPEC (Table 8). One ExPEC virulence-associated factor was present in 13.4% of isolates. *papA*, *iutA*, *papC*, and *kpsMT* II were detected in 9.5, 1.7, 1.3, and 0.9% of generic *E. coli* isolates, respectively.

Prevalence of ExPEC on pre-evisceration carcasses, final carcasses, and strip loins. Colony lysates of generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* from pre-evisceration carcass, final carcass, and strip loin samples were also screened for the presence of ExPEC virulence-associated factors. No colony lysate contained more than one virulence factor. Thus, prevalences of 3GC^r ExPEC, COT^r ExPEC, and generic ExPEC were 0.0% on pre-evisceration carcasses, final carcasses, and strip loins.

Antimicrobial susceptibilities of generic *Salmonella* isolates. Of the 110 generic *Salmonella* isolates examined 79.1% were pansusceptible (Table 7). A total of 20.0% of the generic *Salmonella* isolates were resistant to both FIS and TET, while one isolate (0.9%) was TET resistant.

Antimicrobial susceptibilities and the presence of β-lactamase genes in 3GC^r *Salmonella* isolates. All 37 3GC^r *Salmonella* isolates were resistant to AUG, AMP, TIO, and AXO (Table 7). All 37 isolates were susceptible to CIP, GEN, KAN, NAL, and COT. All isolates but one (97.3%) were resistant to FOX. One isolate was susceptible to FIS and TET. All 37 3GC^r *Salmonella* isolates harbored bla_{CMY}. bla_{CTX-M} was not detected in any 3GC^r *Salmonella* isolate.

Antimicrobial susceptibility of a NAL^r *Salmonella* isolate. The sole NAL^r *Salmonella* isolate was resistant to AMP, CIP, GEN, NAL, and TET but susceptible to AUG, AZI, FOX, TIO, AXO, CHL, KAN, STR, FIS, and COT (Table 7).

DISCUSSION

The contamination of beef carcasses with bacterial populations on the hides during hide removal is termed "hide-to-carcass transfer." The hide-to-carcass transfer of *E. coli* O157:H7 and *Salmonella* has been demonstrated to be a prominent source of final product contamination (27, 29, 47, 48). Since higher pathogen concentrations on hides during processing are correlated with higher rates of beef carcass contamination (29, 49, 50), we investigated here the concentrations and prevalences of AMR *E. coli* and *Salmonella* in beef processing.

Although COT^r *E. coli* was present on all 184 hides at process-

TABLE 5 Prevalences and concentrations of *E. coli* and *Salmonella* on pre-evisceration carcasses

Organism and lot[a]	No. sampled	% prevalence	Frequency of pre-evisceration carcasses with indicated concn (log CFU/100 cm^2)	
			–1.60 to 0.99	1.00–2.00
Generic *E. coli*	184	90.2	138	28
Lot 1	74	100.0	47	27
Lot 2	74	77.0	56	1
Lot 3	36	97.2	35	0
3GC^r *E. coli*	184	2.7	5	0
Lot 1	74	2.7	2	0
Lot 2	74	0.0	0	0
Lot 3	36	8.3	3	0
COT^r *E. coli*	184	32.6	60	0
Lot 1	74	77.0	57	0
Lot 2	74	2.7	2	0
Lot 3	36	2.8	1	0
Generic *Salmonella*	184	2.2	4	0
Lot 1	74	0.0	0	0
Lot 2	74	0.0	0	0
Lot 3	36	11.1	4	0
3GC^r *Salmonella*	184	0.0	0	0
NAL^r *Salmonella*	184	0.0	0	0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.

TABLE 6 Prevalences and concentrations of *E. coli* and *Salmonella* on final carcasses

Organism and lot[a]	No. sampled	% prevalence	Frequency of final carcasses with indicated concn (log CFU/100 cm^2)		
			–1.60 to –0.31	–0.30 to 0.99	1.00–1.99
Generic *E. coli*	184	49.5	26	60	5
Lot 1	74	91.9	3	60	5
Lot 2	74	5.4	4	0	0
Lot 3	36	52.8	19	0	0
3GC^r *E. coli*	184	0.5	1	0	0
Lot 1	74	1.4	1	0	0
Lot 2	74	0.0	0	0	0
Lot 3	36	0.0	0	0	0
COT^r *E. coli*	184	0.5	1	0	0
Lot 1	74	1.4	1	0	0
Lot 2	74	0.0	0	0	0
Lot 3	36	0.0	0	0	0
Generic *Salmonella*	184	0.0	0	0	0
3GC^r *Salmonella*	184	0.0	0	0	0
NAL^r *Salmonella*	184	0.0	0	0	0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.

ing, lot 1 processing hides had a 25.7% incidence of concentrations of COT^r *E. coli* of ≥4.00 log CFU/100 cm^2, higher than the 6.8 and 0.0% incidences for lot 2 and lot 3, respectively (Table 4). Fittingly, COT^r *E. coli* pre-evisceration carcass prevalences were 77.0, 2.7, and 2.8% for lot 1, lot 2, and lot 3, respectively (Table 5). The solitary final carcass contaminated with COT^r *E. coli* was from lot 1 (Table 6).

3GC^r *E. coli* was present on all 184 hides sampled at processing, but the 3GC^r *E. coli* pre-evisceration carcass prevalences were 2.7, 0.0, and 8.3% for lot 1, lot 2, and lot 3, respectively (Tables 4 and 5). Accordingly, concentrations of 3GC^r *E. coli* did not exceed 4.00 log CFU/100 cm^2 on any hide at processing (Table 4). 3GC^r *Salmonella* was not detected on any pre-evisceration carcass or final carcass (Tables 5 and 6). Fittingly, 3GC^r *Salmonella* was detected on only 7.6% of hides during processing, and the hide concentrations were ≥1.60 log CFU/100 cm^2 on only one hide (Table 4).

These results support the existing model of hide-to-carcass transfer and demonstrate the importance of the concentrations of AMR populations present on hides during processing. However, a standardized threshold hide concentration to prevent hide-to-carcass transfer should not be inferred from these results. Contaminant concentration on hides is not the sole factor influencing hide-to-carcass transfer since other factors (proficiency in hygienic hide removal, variation of in-plant interventions, etc.) also impact to hide-to-carcass transfer significantly. For instance, some processing plants with higher hide concentrations of *E. coli* O157:H7 and *Salmonella* on incoming cattle have been demonstrated to have lower rates of hide-to-carcass transfer than plants with lower hide concentrations of *E. coli* O157:H7 and *Salmonella* due to better hide removal technique (29, 49, 51).

Transportation from production environments to the processing plant and the processing plant "lairage" environment (areas cattle pass from arrival at the processing plant until shackling, including holding pens, alleys, and chutes) have been demonstrated to alter *E. coli* O157:H7 and *Salmonella* concentrations on hides between feedlot and processing plant (50, 52). This process has been termed "lairage contamination." It was beyond the scope of this study to irrefutably determine whether lairage contamination was responsible for alterations of AMR bacteria concentrations on hides between feedlot and processing because of study limitations, including the number of days between sampling at feedlots and processing. In addition, sampling lairage environments, including trailers and subtyping isolates were beyond the scope of the present study. Nevertheless, the results, especially for lot 1, were consistent with lairage contamination. When lot 1 cattle were sampled at the feedlot, no hide harbored 3GC^r *E. coli* at concentrations ≥1.60 log CFU/100 cm^2 (Table 2), but at processing, 81.1% of the hides harbored 3GC^r *E. coli* at concentrations ≥1.60 log CFU/100 cm^2 (Table 4). In addition, lot 1 cattle with fecal concentrations of 3GC^r *E. coli* ≥2.00 log CFU/swab dropped from 16.2% at the feedlot to 9.5% at processing (Tables 1 and 3). These observations were consistent with contamination of lot 1 hides with 3GC^r *E. coli* in lairage. Greater alteration of COT^r *E. coli* hide concentrations were observed on lot 1 hides between feedlot and processing. When sampled at the feedlot, only 9.4% of lot 1 hides had COT^r *E. coli* concentrations ≥3.00 log CFU/100 cm^2, and no hide had COT^r *E. coli* concentrations ≥4.00 log CFU/100 cm^2 (Table 2). At processing, 91.9% of lot 1 cattle hides had COT^r *E. coli* concentrations ≥3.00 log CFU/100 cm^2, and concentrations ≥4.00 log CFU/100 cm^2 were detected on 25.7% of hides (Table 4). These observations were consistent with contamination of hides with COT^r *E. coli* in lairage since the occurrence of lot 1 fecal samples with COT^r *E. coli* concentrations ≥3.00 log CFU/swab fell from 41.9 to 24.3% from feedlot to processing (Tables 1 and 3).

TABLE 7 *E. coli* and *Salmonella* isolate susceptibilities to 15 antimicrobial agents

Organism[a]	No. of isolates	% of isolates resistant to[b]:														
		AUG	AMP	AZI	FOX	TIO	AXO	CHL	CIP	GEN	KAN	NAL	STR	FIS	TET	COT
3GC^r *E. coli*	150	64.7	100.0	20.0	64.0	100.0	100.0	69.3	10.7	6.7	4.0	12.7	65.3	68.7	97.3	5.3
COT^r *E. coli*	160	4.4	60.0	2.5	4.4	4.4	4.4	43.8	4.4	3.1	33.8	7.5	72.5	100.0	91.3	100.0
Generic *Salmonella*	110	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	20.9	0.0
3GC^r *Salmonella*	37	100.0	100.0	0.0	97.3	100.0	100.0	29.7	0.0	0.0	0.0	0.0	29.7	97.3	97.3	0.0
NAL^r *Salmonella*	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	0.0	0.0	100.0	0.0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; NAL^r, nalidixic acid resistant.
[b] AMC, amoxicillin-clavulanic acid; AMP, ampicillin; AZI, azithromycin; FOX, cefoxitin; TIO, ceftiofur; AXO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline; COT, trimethoprim-sulfamethoxazole.

3GC^r *Salmonella* is classified in the highest priority of antibiotic resistant food-borne pathogens, as 3rd-generation cephalosporins (CTX, AXO) are the preferred therapies for serious juvenile human *Salmonella* infections (17, 53–57). Brichta-Harhay et al. (58) demonstrated that 3GC^r *Salmonella* spp. were a subpopulation of generic *Salmonella* in beef processing environments using extensive antimicrobial susceptibility analysis of generic *Salmonella* isolates (i.e., isolates of colonies grown on media permissive for *Salmonella* regardless of antimicrobial susceptibility), but the concentrations of 3GC^r *Salmonella* subpopulations could not be determined by Brichta-Harhay et al. since *Salmonella*-specific media supplemented with third-generation cephalosporins were not used to specifically enumerate 3GC^r *Salmonella*.

In the present study, samples were plated onto XLD+CTX plates to enumerate 3GC^r *Salmonella*, but enumerable levels were present in only two samples. RVS secondary enrichment cultures were plated on XLD+CTX to improve detection when 3GC^r *Salmonella* concentrations were below the lower limit of enumeration. 3GC^r *Salmonella* was detected in 38 samples, all of which were hide or fecal samples. The absence of 3GC^r *Salmonella* contamination of pre-evisceration carcasses, final carcasses, and strip loins was likely because the concentrations of 3GC^r *Salmonella* subpopulations on the hides at processing were at levels low enough to make hide-to-carcass transfer unlikely. However, the results demonstrated that the methods utilized here improved the detection of 3GC^r *Salmonella* on hides and in feces when concentrations were not enumerable. All 110 generic *Salmonella* isolates subjected to broth microdilution susceptibility testing were susceptible to both third-generation cephalosporins (AXO and TIO) tested, including 10 generic *Salmonella* isolates obtained from samples (7 hide, 3 fecal) that were positive for 3GC^r *Salmonella*.

Fluoroquinolones are the preferred therapy for adults with serious *Salmonella* infections, and fluoroquinolone-resistant (FQ^r) *Salmonella* is classified in the highest priority of antimicrobial-resistant food-borne bacteria (17, 53–57). While FQ^r *Salmonella* are rarely isolated from U.S. and European clinical infections and meat products, FQ^r *Salmonella* are more frequently isolated outside the United States and Europe (30, 59–62). Culture and isolation of NAL^r *Salmonella* effectively monitor emerging FQ^r *Salmonella* since NAL^r *Salmonella* commonly has a single point mutation in *gyrA*, while FQ^r *Salmonella* has additional point mutations in *gyrA* (63–65). Reduced susceptibility to fluoroquinolones is frequently observed with NAL^r *Salmonella*, and NAL^r *Salmonella* infections have been reported in the United States (63, 65–67). However, NAL^r *Salmonella* was detected in only one sample in the present study, but the NAL^r *Salmonella* isolate from this sample was also CIP resistant.

In the United States, 3GC^r *E. coli* has been isolated from animal, food, and human sources (30). Commensal 3GC^r *E. coli* in cattle feedlots represents a subpopulation of the generic *E. coli* hide and fecal populations (36, 68–70). Resistance to third-generation cephalosporins in both *E. coli* and *Salmonella* is commonly conferred by a cephamycinase encoded by a bla_{CMY} gene harbored on a conjugative plasmid (15, 30, 71–73). The nucleotide sequences of bla_{CMY} bearing conjugative plasmids harbored by *E. coli* and *Salmonella* are conserved (72, 73). Thus, *E. coli* in animals, foods, and humans is theorized to be a reservoir of 3GC^r capable of transfer to pathogens, including *Salmonella* (15, 74–82). *Salmonella* and *E. coli* resistance to third-generation cephalosporins may be conferred by a CTX-M type beta-lactamase, as well, encoded by $bla_{CTX\text{-}M}$. *Salmonella* with $bla_{CTX\text{-}M}$ is rarely isolated in the United States, but increasing reports of $bla_{CTX\text{-}M}$ 3GC^r *E. coli* isolated from food animal feces have raised fears that $bla_{CTX\text{-}M}$ 3GC^r *Salmonella* may emerge in the United States (45, 71, 83, 84). In the present study, the $bla_{CTX\text{-}M}$ gene was detected from 36.7% of 3GC^r *E. coli* isolates tested but from none of the 3GC^r *Salmonella* isolates tested. Conversely, the bla_{CMY} gene was detected in 63.3% of 3GC^r *E. coli* isolates and all 3GC^r *Salmonella* isolates. We conclude that in the examined environments $bla_{CTX\text{-}M}$ *E. coli* was not a reservoir of $bla_{CTX\text{-}M}$ for *Salmonella*.

Sulfamethoxazole and trimethoprim inhibit synthesis of tetrahydrofolate at two different steps. Accordingly, human clinical COT^r *E. coli* isolates typically harbor at least one *sul* gene encoding a dihydropteroate synthase insensitive to sulfonamide and at least

TABLE 8 Detection of ExPEC virulence-associated markers in *E. coli* isolates[a]

Organism	No. of isolates	% ExPEC	% of isolates with indicated marker					
			afa/dra	*iutA*	*kpsMT* II	*papA*	*papC*	*sfa/foc*
Generic *E. coli*	232	0.0	0.0	1.7	0.9	9.5	1.3	0.0
3GC^r *E. coli*	150	0.0	0.0	0.7	0.0	0.0	0.0	0.0
COT^r *E. coli*	160	1.3	0.0	50.6	0.6	0.0	1.3	0.0

[a] 3GC^r, third-generation cephalosporin resistant; COT^r, trimethoprim-sulfamethoxazole resistant; ExPEC, extraintestinal pathogenic *E. coli*.

one *dfrA* gene encoding a dihydrofolate reductase insensitive to trimethoprim (46, 85–88). At least one *sul* gene was detected in 99.4% of the 150 COT^r *E. coli* isolates examined here. Prevalences of *sul1*, *sul2*, and *sul3* were 70.6, 39.4, and 22.5%, respectively. This pattern of *sul* gene frequencies contrasted with the pattern observed in most studies of human COT^r *E. coli* isolates: *sul2* > *sul1* > *sul3* (86, 87, 89, 90). However, the pattern *sul1* > *sul2* > *sul3* has been observed in at least one study of human COT^r *E. coli* isolates (88). At least one of four common *dfrA* genes (*dfrA1*, *dfrA5*, *dfrA7*/*dfrA17*, and *dfrA12*) was detected in 91.3% of the COT^r *E. coli* isolated in the present study. The predominate gene was *dfrA5*, detected from 51.3% of isolates, which contrasted with the predominance of *dfrA1* and *dfrA17* in human COT^r *E. coli* isolates (46).

E. coli causes 75 to 95% of human UTIs in the United States (22). ExPEC differ from both commensal *E. coli* and Shiga-toxigenic *E. coli* in their phylogeny and virulence factors (32, 91–93). In the present study, 0.0, 0.0, and 1.3% of generic *E. coli*, 3GC^r *E. coli*, and COT^r *E. coli* isolates were ExPEC, respectively (Table 8). Similarly Xia et al. (21) determined that 3.4% of 293 *E. coli* isolates from retail ground beef were ExPEC. In addition, we found that ExPEC were not present on any pre-evisceration carcass, final carcass, or strip loin. A literature review by Nordstrom et al. (18) put forth the opinion that meat products (including ground beef) were a source of ExPEC that cause human UTIs. Our findings suggest that beef cattle and beef products are not significant sources of ExPEC causing human UTIs. However, stronger conclusions will require a broader data set than that examined here.

In summary, we clearly demonstrated that AMR subpopulations of *E. coli* and *Salmonella* exist on the hides and in the feces of beef cattle both at feedlots and during processing. Moreover, we demonstrated that COT^r *E. coli* and 3GC^r *E. coli* concentrations on hides may change between feedlot and processing, a finding consistent with the "lairage contamination" model for *E. coli* O157:H7 and *Salmonella*. Enumeration of COT^r *E. coli* and 3GC^r *E. coli* subpopulations on hides during processing determined that these concentrations were correlated to carcass contamination, analogous to the results of *E. coli* O157:H7 and *Salmonella* studies examining hide-to-carcass contamination. The present study thus demonstrated that currently used processing interventions are effective for AMR bacteria since 3GC^r *E. coli* and COT^r *E. coli* were present on 100% of hides when processing began (Table 4) but the prevalences of 3GC^r *E. coli* and COT^r *E. coli* on final carcasses were each 0.5% (Table 6).

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